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THE POSSIBILITY OF CONTROLLING THE MUTATION PROCESS BY USING CHEMICAL MUTAGENS REACTING PREFERENTIALLY WITH SINGLE-STRAND DNA IN CONJUNCTION WITH LOCAL CHANGES IN THE STATE OF CELLULAR DNA

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Earlier, we postulated that the higher sensitivity of single-strand or denaturated segments of DNA to chemical mutagens can be used to involve discrete genes in the mutation process and obtain limited mutation spectra in conjunction with local denaturation of cellular DNA (1, 2).

In a number of investigations carried out in our laboratory, it was shown that denaturated DNA does in fact react much more intensely than native DNA with specific chemical mutagens (3,4). Thus, in the reaction with hydroxylamine, the cytosine loss is 32.6 ± 1.6 in denaturated DNA and only 10.1 ± 3.2% in native DNA (3); denaturated DNA adds 2.5 -- 5.6 times as much of the chromophoric derivative of nitrogen mustard as native DNA does (4); denaturated DNA also reacts much more intensely with dimethyl sulfate (5).

Denaturation of different but discrete segments of DNA can occur as a result of the action of certain external or internal (cell) factors. Thus, it is known that DNA is denaturated by UV irradiation and that the number or size of the denaturated segments increases with the UV doses (6).

The secondary structure of DNA can be disrupted as a result of the physiological process of its replication; if the replication wave travels along the DNA molecule, at different time intervals in the course of this process, different DNA segments can exhibit increased sensitivity to chemical mutagens which react preferentially with single-strand DNA.

Table 1.

Ratio of nitrogen bases in the native portion of DNA and in the UV ray-denaturated fraction.

DNA Characteristic	(A + T) / (G + C)
Entire DNA	1.33 ± 0.05
UV-denaturated DNA fraction	1.8 ± 0.17
Native DNA portion	1.14 ± 0.1

It was postulated that by applying "swift blows" with chemical mutagens to such sensitive DNA segments, it will be possible to involve discrete genes in the mutation process.

In the past few years, we verified experimentally the main propositions of this scheme. To this end, as was indicated above, we studied the interaction of a number of chemical mutagens with native and with denaturated DNA (3, 6). The capacity of UV rays to cause DNA denaturation was also studied (7, 9) and it was shown that UV denaturation affects first of all DNA regions which are richest in adenine -- thymine (A -- T) base pairs (9). We used a method which makes it possible to isolate from the DNA molecule as a whole those segments which have undergone denaturation (7, 8). This method involves selective chemical modification of denaturated DNA segments by water-soluble carbodiimide and subsequent fermentative hydrolysis of nonmodified native segments. In the form of oligonucleotide blocks, denaturated segments are separated by gel filtration from the mononucleotides which reflect the amount and composition of the native portion of the DNA (8, 9). As Table 1 indicates, the A -- T pair content of the UV-denaturated DNA segments is 1.6 times as high as that of the native portion.*

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*DNA from calf thymus was subjected to UV irradiation ($1.5 \cdot 10^6$ erg/mm^2 ; the denaturated portion was separated by gel filtration after treatment with water-soluble carbodiimide and hydrolysis with deoxyribonuclease with snake-poison phosphodiesterase. The nitrogen base ratio was calculated from the thymine and guanine content (A -- T; G -- C [sic]) in conformity with the method in reference (1).

Table 2.

Chloroform fractionation of DNA from normal and regenerating liver.

Source of DNA	Number of Experiments	DNA_{denat} in interphase layer, %
Normal Rat Liver	10	3.5 ± 1.5
Regenerating Rat Liver		
12 -- 17 hr.	10	6.7 ± 3.4
22 -- 25 hr.	13	15.6 ± 3.7
45 -- 50 hr.	8	7.2 ± 2.4

Evidently, under the action of UV rays, denaturation does not take place at random, but in each DNA molecule discrete segments rich in A -- T pairs are the first to be attacked; as the UV ray dose increases, new DNA segments less rich in A -- T pairs must evidently become vulnerable to the action of chemical mutagens. In recent years, a number of investigations have shown that the DNA of rapidly dividing bacterial cells, tissue cultures, and cancer cells contains fractions which exhibit certain properties inherent in denaturated DNA (10, 11). Such data were also obtained in our experiments (12, 13). It is known that most cells of regenerating liver, 18 -- 20 hr. after partial hepaectomy, enter an S period which lasts for up to 26 -- 28 hr. Comparison of the state of DNA isolated during the replicative period and in the pre- and post-replicative periods showed that in the period of replication the content of the fraction exhibiting the properties of denaturated DNA increases from 7 to 15% (Table 2). We isolated such DNA by fractionation in a chloroform/water two-phase system (12), as well as by chemical modification with carbodiimide with subsequent fermetative hydrolysis and gel filtration (12). Data on the inclusion in these fractions of thymidine-C^{14}

suggested that unpaired segments are present both in prereplicative and in early postreplicative DNA (13). Thus, these results indicate that replication is accompanied by the rupture of hydrogen bonds in matrix DNA in autoduplication segments.

The above ideas and the sum of the above data served as a basis for genetic experiments. Spectra of auxotrophic mutants of the prototrophic strain of E. coli B were investigated on simultaneous action of UV rays and chemical mutagens. The UV ray dosages used (100 and 200 erg/mm^2) had no mutagenic effect under the conditions of the experiments. The experimental procedure was described in detail in reference (14). Under the effect of hydroxylamine alone (0.3 M solution), auxotrophs deficient in phenylalanine--alanine appeared.

If after addition of hydroxylamine, the bacterial culture was irradiated with a nonmutagenic dose of UV rays (100 erg/mm^2), the auxotrophic mutant spectrum widened and additional auxotrophs deficient in proline and glycine appeared; further widening of the auxotrophic spectrum was brought about by the action of hydroxylamine followed by irradiation with a dose of 200 erg/mm^2.

In genetic experiments with a synchronized E. coli culture, the possibility was investigated of involving different genes in the mutation process by applying "swift blows" with a chemical mutagen which reacts with single-strand DNA in various replication periods (15). The introduction of such mutagens during various periods of the lag phase (synchronous replication of DNA) did in fact prove to cause the appearance of different and quite specific mutations in each period. Thus, in a 15-minute incuba-

Fig. 1. Spectrum of mutants obtained through the action of formaldehyde during various periods of E. coli chromosome replication in a synchronized culture.
Legend: a. Number of auxotrophic mutants; b. min; c. min; d. markers of spread-out chromosome.

tion of bacterial cells with formaldehyde at the beginning of a lag phase, it is mostly mutants deficient in proline which appear. On incubation of the bacteria with formaldehyde in the middle of the lag phase (from the 30th to the 45th min) the number of proline mutants drops, but a large number of auxotrophs deficient in purines, as well as mutants deficient in histidine appear. On incubation of the bacteria with formaldehyde at the end of the lag phase (from the 60th min on) the number of histidine-deficient mutants rises to 59% and that of purine-deficient mutants drops (Fig.1). A similar pattern is observed on incubation of a synchronized E. coli culture with hydroxylamine (15).

Specific mutations can also be brought about by introducing during different DNA replication periods, base analogs which will induce inclusion errors in various loci of the DNA of synchronously dividing bacterial culture. Such a possibility was shown by Ryan and Cetrullo who established that if 2-aminopurine is introduced at the beginning of the E. coli replication cycle, the frequency of reverse mutations of the strain deficient in guanine is high, but that if 2-aminopurine is introduced later, the frequency of reverse mutations in this locus does not exceed the level of spontaneously occuring mutations (16).

It can be postulated that these principle of generating specific mutations are applicable to various cell populations in which the processes of cell division (and, therefore, DNA replication) can be synchronized.

The data obtained in this research confirm ideas on the possibility of influencing the mutation spectrum by using specific chemical mutagens which react preferentially with denaturated DNA in conjunction with the action of factors capable of locally inducing the DNA denaturation process.

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